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DETECTION OF CHROMOSOMAL DEFECTS IN MORPHOLOGICALLY NORMAL AND ABNORMAL 4-DAY EMBRYOS OF MICE USING MULTI-PROBE FLUORESCENCE IN SITU HYBRIDIZATION (FISH) T. Ahlborn[1,2]*, X. Lowe[1], N. Titenko-Holland[2], M. Smith[2], and A. J. Wyrobek[1] [1]BBRP, LLNL, Livermore, CA; [2]SPH, University of California, Berkeley, CA

Inherited cytogenetic abnormalities can cause failure of early cleavage divisions and lead to abnormal embryonic development. We are developing new methods to detect morphological and molecular cytogenetic defects in preimplantation mouse embryos (4-day p.c.) using multi-probe FISH. Embryos, flushed from the uterine horns, were evaluated under a stereo microscope and assigned to various categories of developmental arrest and morphological degradation. Three types of morphological abnormalities were noted: 1) arrest of cleavage, 2) embryos with lysis of blastomere(s), and 3) oocytes that were not fertilized and/or zygotes which failed to undergo cleavage. The embryos were prepared with a two step fixation process employing combinations of methanol and acetic acid. Individual embryos were evaluated using FISH with a variety of DNA probes. The Y and centromeric probes were labeled directly with FITC and Cy3, respectively, while the X chromosome probe was labeled with digoxigenin and detected with rhodamine conjugated anti-digoxigenin antibodies. This combination of DNA probes enabled the analysis of both interphase and metaphase cells of the embryo and the study of three cytogenetic endpoints: micronucleus formation, polyploidy, and aneuploidy of the sex chromosomes. Micronuclei were detected using DAPI and characterized with DNA probes, while the overall ploidy and the sex chromosomal ploidy of each embryo were also examined. These methods have applications in studies of genetic factors affecting embryo susceptibility, embryo toxicology, and for determining the developmental consequences of genetic damage transmitted via maternal or paternal germ cells.

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